UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: December 1st, 2015

SUBJECT: **FLUFENACET** – Supplemental Data Evaluation Record (DER) for the comparative thyroid studies.

PC Code: 121903	**DP Barcode:** D430198
Decision No.: NA	**Registration No.:** NA
Petition No.: NA	**Regulatory Action:** NA
Risk Assessment Type: NA	**Case No.:** NA
TXR No.: 0057310	**CAS No.:** 142459-58-3
MRID No.: 48898902-04, 49415601	**40 CFR:** NA

Ver.Apr. 2010

FROM: Minerva Mercado-Feliciano Ph.D. DABT, Toxicologist
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THROUGH: Christine Olinger, Branch Chief
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TO: Margaret Hathaway, Chemical Review Manager
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I. CONCLUSIONS

HED has revised the Data Evaluation Records (DER) for a suite of three comparative thyroid studies for flufenacet technical. All three studies considered together are classified as **unacceptable** for risk assessment purposes. However they satisfy the conditions of registration and data requirement.

EPA Reviewer: Minerva Mercado-Feliciano Ph.D., DABT **Signature:**
RAB III, Health Effects Division (7509P) **Date:** 11.24.2015
EPA Secondary Reviewer: Elissa Reaves Ph.D. **Signature:**
Immediate Office, Health Effects Division (7509P) **Date:** 12/1/15
Template version 03/12

TXR#: 0057310

DATA EVALUATION RECORD – Supplemental

See TXR #0057198 for root DER

This supplement contains an amended Executive Summary and Critical Findings Tables to remove the NOAEL/LOAEL statement and update the study classification based on the current evaluation policy.

STUDY TYPE: Comparative Thyroid Assay - Rat; Non-guideline

PC CODE: 121903 **DP BARCODES:** D430198

TEST MATERIAL (PURITY): Flufenacet (96.8%)

SYNONYMS: FOE5043

CITATION: Blanck, M (2012) Flufenacet (FOE5043) Comparative Thyroid Sensitivity Assay in the Rat (Gestational Exposure Phase). Bayer S.A.S (Sophia Antipolis, France). Laboratory report number SA10154, July 27, 2012. MRID 48898902. Unpublished.

Blanck, M (2012) Flufenacet (FOE5043) Comparative Thyroid Sensitivity Assay in the Rat by Dietary Exposure (Gestational and Lactational Exposure Phase). Bayer S.A.S (Sophia Antipolis, France). Laboratory report number SA11052, July 27, 2012. MRID 48898903. Unpublished.

Blanck, M (2012) Flufenacet (FOE5043) Comparative Thyroid Sensitivity Assay in the Rat Complementary Assay (Gavage Exposure of Pups). Bayer S.A.S (Sophia Antipolis, France). Laboratory report number SA11167, July 25, 2012. MRID 48898904. Unpublished.

Blanck, M (2015) Flufenacet Comparative Thyroid Sensitivity Studies: Response to Questions from US EPA. Bayer CropScience AG (Monheim, Germany). Laboratory report number US0459, January 8, 2015. MRID 49415601. Unpublished.

SPONSOR: Bayer CropScience AG, Monheim, Germany.

EXECUTIVE SUMMARY:

In a battery of 3 comparative thyroid assays (MRIDs 48898902, 48898903 and 48898904), flufenacet (96.8% a.i., lot# NK61AX0177) was administered to Sprague-Dawley Crl:CD(SD) rats at different developmental stages via diet at concentrations of 0, 20, 100 or 500 ppm (gestation-only assay and gestation/lactation assay: or via gavage at doses of 0 or 1.7 mg/kg/day

(pup gavage assay). The thyroid peroxidase inhibitor, 6-propyl-2-thiouracil (PTU), was administered in the diet as a positive control pregnant female rats at 15 ppm (0.9 mg/kg/day).

In the gestation-only assay (MRID 48898902), 10 pregnant rats per group received flufenacet in the diet, equivalent to 0, 1.3, 6.8 or 34.8 mg/kg/day, from gestation day (GD) 6 through gestation day 20. Daily clinical observations and GD 3, 6, 13 and 20 maternal body weights and cumulative food consumption were recorded. On GD 20, blood samples were collected (individual dams and whole litter pooled fetuses) and analyzed for serum thyroid hormones (T3, T4 and TSH); thyroid weight and thyroid histopathology were also evaluated (individual dams and litter averages for fetuses).

In the gestation/lactation assay (MRID 48898903), 20 pregnant rats/group received flufenacet in the diet, equivalent to 1.3, 6.6 and 35.2 mg/kg/day from GD 6 through 20 (gestation period) and 3.4, 16.7, or 84.2 mg/kg/day during lactational period from post-natal day or lactation day (LD) 4 to 21. The dose levels during the lactation period were about 2.5X of those during the gestation period. Parameters evaluated in dams and pups included clinical observations, detailed physical examinations, body weight, and food consumption (per cage). On LD 4 (interim sacrifice, N = 5) and LD 21 (terminal sacrifice, N = 15), serum samples were collected from all groups for analysis of hormone (T3, T4, TSH) levels. Thyroid weight was measured and thyroid histopathology evaluated in dams and pups at terminal sacrifice.

In the pup gavage assay (MRID 48898904), 15 pups/sex/group from untreated dams (one male and female within each litter) were dosed at 0 or 1.7 mg/kg/day by oral gavage from LD 10 through LD 20. On LD 21, serum levels of T3, T4 and TSH and terminal body and thyroid weights were measured. Histopathology was performed on the thyroid gland.

Flufenacet had no adverse effect on dam survival, body weights or liver parameters. Thyroid atrophy was noted grossly in gestating dams treated with the low and mid doses of flufenacet, however not at the high dose. Microscopically, incidence of intrafollicular debris was observed in lactating dams in a non-dose-dependent pattern similar to the incidence pattern of gross atrophy in gestating dams. The intrafollicular debris findings in the gestational and lactational dams were not considered as adverse. However, 1/14 and 2/13 dams in the mid and high dose, respectively, of the gestational/lactational exposure study presented follicular cell hypertrophy; this finding appeared to be treatment-related because none was seen in the controls (0/15).

Thyroid hormone results varied by treatment phase as well as by the sensitivity of the assay. The T4 assay was based on the manufacturer's level of quantification (LOQ) of 10 ng/mL. T4 levels were reduced in lactating dams at the mid (26%; 16.9 ± 4.0 ng/mL) and high dose (55%; 10.3 ± 0.6 ng/mL) compared to controls (22.9 ± 4.3 ng/mL) but not at the low dose (23.6 ± 4.4 ng/mL). In gestating dams, mean T4 levels were observed to decrease in a dose-dependent manner, however the changes were not statistically different from control (19.1 ± 8.3 ng/mL) and at the high dose mean T4 levels were close to the LOQ (12.0 ± 6.5 ng/mL). Mean T4 levels in control and flufenacet-treated dams were above the LOQ for the assay during gestation and lactation, however T4 levels in PTU-treated dams could not be quantified (were below the LOQ). T3 levels were decreased in lactating (but not gestating) dams only at the high dose. TSH was decreased (13-23%) in all dose groups on GD 20 but increased in all dose groups (38-95%) on LD 21. The TSH changes were not dose-dependent and/or statistically significant and did not show the expected correlation with T4 measurements. PTU treated animals did show the expected increase in TSH

(increased 331% compared to controls) with decreased T4 levels (in this case T4 was below the LOQ).

In summary, lactating dams exposed from GD 6 to PND 21, had dose-dependent decreases in mean T4 and T3 levels and increased incidence of thyroid follicular cell hypertrophy after exposure to 100 ppm or 500 ppm flufenacet in the diet. The status of TSH levels was not consistent in maternal animals treated with flufenacet, specifically since lactating dams displayed dose-dependent non-statistically significant increases in serum while gestating dams displayed dose-dependent non-statistically significant decreases in serum TSH.

Flufenacet had no effect on the viability or fetal body weight of offspring exposed during gestation and lactation. In lactating pups, body weights were decreased 13-22% at the high dose. On LD 7 and 14 pup mean body weights also seem to be reduced (2-6%) in a dose-dependent manner, however the differences were not statistically significant.

Fetal TSH levels were decreased in all groups in a dose-dependent manner (19-45%), and showed statistical significance at the mid and high dose levels. T4 levels could not be properly assessed in fetuses or LD 4 pups because most individual measurements were below or at the LOQ. In LD 21 pups, T4 levels did not change with treatment, however T3 levels were decreased at the mid and high. TSH levels did not change from control in LD 4 or LD 21 pups treated with flufenacet.

In summary, offspring exposed perinatally to flufenacet (GD 6-LD 21) resulted in a dose-dependent decrease in mean serum TSH levels on GD 20, a dose-dependent decrease in mean serum T3 levels on PND 21, and decreased mean pup body weight (2-22%) during lactation at all doses, although only statistically significant at the high dose. T4 levels could not be assessed in offspring for most groups treated with flufenacet.

No effect of flufenacet (no change in body weights, TSH, T3 or T4) was observed in pups of untreated dams that were dosed directly with 1.7 mg/kg/day of flufenacet by gavage on PND 10-20 (no gestation or lactation exposure). Mean T4 levels in control and flufenacet-treated pups were above the LOQ for the assay.

In the current studies, the positive control, 6-propyl-2-thiouracil (PTU), produced the expected effects. However, T4 levels could not be fully assessed in flufenacet exposed offspring, and serum TSH in dams and pups displayed unexpected trends, making the results of this study difficult to interpret. Therefore, these non-guideline studies are not acceptable and should not be used quantitatively in risk assessment. Although there is no official guidance for conducting this type of study, a preliminary guidance document exists and was referenced by the investigator (Guidance for Thyroid Assays in Pregnant Animals, Fetuses and Postnatal Animals and Adult Animals. Office of Pesticide Programs, Health Effects Division, Washington DC; October 24, 2005). All three studies considered together are classified as **unacceptable** due to the lack of reliable T4 measurements in offspring and lactating dams.

Critical data tables from the original DER are copied in the next page.

TABLE 10. Mean (± SD) pup body weights (g) during lactation treatment period [a]

Lactation day	Flufenacet (ppm)				PTU 15 ppm
	0	20	100	500	
mg/kg/day	0	3.4	17	84	1.9
Males					
4 [b]	11.6 ± 1.4	11.1 ± 0.8	11.2 ± 1.2	9.3 ± 1.4 ** (↓20)	9.7 ± 1.4 ** (↓16)
7	18.9 ± 2.0	18.1 ± 1.1	18.1 ± 1.4	14.8 ± 2.5 ** (↓22)	15.0 ± 1.9 ** (↓21)
14	37.5 ± 2.9	36.8 ± 3.4	36.6 ± 3.1	31.4 ± 4.0 ** (↓16)	26.6 ± 2.7 *** (↓29)
21	57.7 ± 5.0	55.3 ± 4.7	57.5 ± 6.3	48.5 ± 5.8 ** (↓16)	32.9 ± 2.2 ** (↓43)
Females					
4 [b]	11.1 ± 1.4	10.3 ± 1.0	10.5 ± 1.2	9.0 ± 1.3 ** (↓19)	9.3 ± 1.3 ** (↓16)
7	18.0 ± 2.1	17.4 ± 1.4	16.9 ± 1.2	14.4 ± 2.5 ** (↓20)	14.3 ± 1.9 ** (↓21)
14	36.5 ± 3.5	35.6 ± 4.0	34.9 ± 3.2	31.2 ± 3.7 ** (↓15)	25.8 ± 3.2 ** (↓29)
21	54.0 ± 4.3	52.7 ± 5.3	54.4 ± 6.5	46.8 ± 5.3 ** (↓13)	32.1 ± 2.9 ** (↓41)

[a] Data obtained from pages 128-130 in the study report (MRID 48898903).
[b] After standardization (culling).
( ): % change from control. Statistically different from control: * $p<0.05$; ** $p<0.01$

Table 17. Summary Table for Comparison of Maternal vs Offspring Hormone Levels, mean ng/mL ± SD

Observation		Flufenacet Dose (ppm)			
		0	20	100	500
Gestation-Only Assay					
Maternal GD 20	T3	1.08 ± 0.11	1.07 ± 0.12	1.05 ± 0.22 (↓3)	0.98 ± 0.09 (↓9)
	T4	19.1 ± 8.3	16.2 ± 5.9 (↓15)	16.6 ± 5.3 (↓13)	12.0 ± 6.5 (↓37)
	TSH	2.20 ± 1.63	1.92 ± 0.55 (↓13)	1.82 ± 0.61 (↓17)	1.69 ± 0.55 (↓23)
GD 20 Fetus pooled litters	T3	0.56 ± 0.07	0.57 ± 0.05	0.55 ± 0.06	0.54 ± 0.04
	T4	< LOQ	< LOQ	< LOQ	< LOQ
	TSH	4.49 ± 1.27	3.65 ± 0.48 (↓19)	3.17 ± 0.61**(↓29)	2.47 ± 0.50** (↓45)
Gestation/Lactation Assay					
Maternal LD 21	T3	0.73 ± 0.09	0.71 ± 0.16 (↓3)	0.67 ± 0.14 (↓8)	0.59 ± 0.11* (↓19)
	T4	22.9 ± 4.3	23.6 ± 4.4	16.9 ± 4.0** (↓26)	10.3 ± 0.6** (↓55)
	TSH	1.37 ± 1.01	2.28 ± 1.74 (↑66)	1.89 ± 1.15 (↑38)	2.67 ± 2.66 (↑95)
LD 4 Pup pooled litters	T3	0.71 ± 0.14	0.72 ± 0.14	0.76 ± 1.14	0.72 ± 0.16
	T4	10.0 ± 2.3 [d]	10.0 ± 2.2 [d]	< LOQ [e]	10.5 ± 3.6
	TSH	1.51 ± 0.55	1.52 ± 0.77	2.96 ± 4.13	1.24 ± 0.44
LD 21 Pup males	T3	1.10 ± 0.20†	0.94 ± 0.22 (↓15)	0.92 ± 0.15* (↓16)	0.84 ± 0.14** (↓24)
	T4	31.8 ± 4.6	33.0 ± 9.8	31.6 ± 6.7	34.1 ± 8.6
	TSH	0.78 ± 0.51	0.86 ± 0.42	1.17 ± 1.21	0.79 ± 0.56
LD 21 Pup females	T3	1.24 ± 0.18+	1.14 ± 0.19 (↓8)	1.16 ± 0.15 (↓6)	1.04 ± 0.18* (↓16)
	T4	30.9 ± 6.7	33.6 ± 10.9	30.3 ± 5.5	33.3 ± 5.4
	TSH	1.24 ± 0.42	1.40 ± 0.76	1.62 ± 1.09	1.22 ± 0.59
Subchronic Study (MRID 43743401) N=15					
ppm →		**0**	**NA**	**100**	**400**
Females 84 days exposure	T3	0.9 ± 0.1	--	0.9 ± 0.2	0.9 ± 0.2
	T4	39 ± 0.8	--	34 ± 0.6	32 ± 0.6* (↓14)
	TSH	NA	--	NA	NA

LOQ = limit of quantification = 0.25 for T3, 10 for T4, 0.5 for TSH (ng/mL). *: $p<0.05$; **: $p<0.01$. ( ) % relative to controls.